

Benperidol

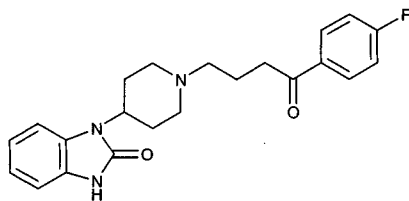
Molecular formula: C₂₂H₂₄FN₃O₂

Molecular weight: 381.45

CAS Registry No.: 2062-84-2

Merck Index: 1077

Lednicer No.: 2 290



SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 246

CHROMATOGRAM

Retention time: 4.62

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylcegonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loperazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demoxiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui, A.; Kintz, P.; Mangin, P. Systematic toxicological analysis using HPLC/DAD, *J. Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.8

OTHER SUBSTANCES

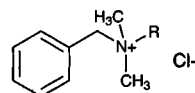
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, peri-

cyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191-225.

Benzalkonium chloride



CAS Registry No.: 8001-54-5

Merck Index: 1086

R = alkyl

SAMPLE

Matrix: formulations

Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN

Mobile phase: MeCN:buffer 50:50 (Buffer was 13.6 g/L sodium acetate, pH adjusted to 4.5 with glacial acetic acid.)

Flow rate: 1.5

Detector: UV 254

CHROMATOGRAM

Retention time: 8 (C12), 11 (C14), 14 (C16)

OTHER SUBSTANCES

Simultaneous: demecarium

KEY WORDS

ophthalmic solution

REFERENCE

Cohn, L.J.; Greeley, V.J.; Tibbetts, D.L. Determination of demecarium bromide and related compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, 321, 401–405.

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 300 × 3.9 10 μm LiChrosorb Si-60

Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 6.0

Flow rate: 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: domiphen bromide, thimerosal, xylometazoline

KEY WORDS

nasal drops

REFERENCE

Lingeman, H.; van Munster, H.A.; Beynen, J.H.; Underberg, W.J.; Hulshoff, A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, 352, 261–274.

SAMPLE**Matrix:** formulations**Sample preparation:** Condition a C18 Sep-Pak SPE cartridge with THF:mobile phase 70:30 then with water. Add 10 mL formulation to the SPE cartridge, wash with water, elute with 3 mL THF:mobile phase 70:30 then with 3 mL mobile phase, mix the eluates, inject a 100 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Zorbax Stablebond CN**Mobile phase:** THF:water:triethylamine 150:250:2, pH adjusted to 3.0 \pm 0.1 with phosphoric acid**Column temperature:** 40**Flow rate:** 2**Injection volume:** 100**Detector:** UV 215

CHROMATOGRAM**Retention time:** 3.5 (C12), 6.5 (C14), 10 (C16)

KEY WORDSSPE; eye

REFERENCE

Elrod,L.,Jr.; Golich,T.G.; Morley,J.A. Determination of benzalkonium chloride in eye care products by high-performance liquid chromatography and solid-phase extraction or on-line column switching, *J.Chromatogr.*, **1992**, 625, 362–367.

SAMPLE**Matrix:** formulations**Sample preparation:** Condition a 1 mL Supelcoclean cyano SPE cartridge with 2 mL MeCN and 2 mL water. Add 4 mL formulation to the SPE cartridge, wash with 2 mL MeCN:buffer 30:70, elute with 5 mL mobile phase, make up eluate to 10 mL with water, inject a 100 μ L aliquot. (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.).

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Ultrasphere cyano**Mobile phase:** MeCN:buffer 60:40 (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.)**Flow rate:** 2**Injection volume:** 100**Detector:** UV 210

CHROMATOGRAM**Retention time:** 4.5 (C10), 5.5 (C12), 6.5 (C14), 7.5 (C16), 8.5 (C18)

OTHER SUBSTANCES**Also analyzed:** tyloxapol

KEY WORDSophthalmic solutions; eye; SPE

REFERENCE

Fan,T.Y.; Wall,G.M. Determination of benzalkonium chloride in ophthalmic solutions containing tyloxapol by solid-phase extraction and reversed-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1993**, 82, 1172–1174.

SAMPLE

Matrix: formulations

Sample preparation: 2 mL Sample + 1 mL 200 µg/mL emetine hydrochloride in water, make up to 10 mL with mobile phase, filter (0.45 µm), inject a 50-100 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 µm Technosphere RP C-8 (HPLC Technology)

Mobile phase: MeCN:40 mM tetramethylammonium bromide:1 M acetic acid 80:15:5 (apparent pH 4.5)

Flow rate: 1.5

Injection volume: 50-100

Detector: UV 260

CHROMATOGRAM

Retention time: 3.15 (C12), 4.21 (C14), 5.78 (C16)

Internal standard: emetine (1.75)

Limit of quantitation: 10 µg/mL

OTHER SUBSTANCES

Simultaneous: naphazoline, tetrahydrozoline

KEY WORDS

nasal; ophthalmic

REFERENCE

Santoni,G.; Medica,A.; Gratteri,P.; Furlanetto,S.; Pinzaui,S. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions, *Farmaco*, **1994**, 49, 751-754.

SAMPLE

Matrix: formulations

Sample preparation: Inject a 50 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak phenyl

Mobile phase: MeCN:buffer 65:35 (Buffer was 50 mM KH₂PO₄ and 57 mM sodium hexanesulfonate, adjusted to pH 6.3 with 1 M NaOH.)

Flow rate: 1.8

Injection volume: 50

Detector: UV 215

CHROMATOGRAM

Retention time: 7.5 (C12), 12.1 (C14)

OTHER SUBSTANCES

Noninterfering: phenylephrine

KEY WORDS

ophthalmic solution; stability-indicating

REFERENCE

Parhizkari,G.; Miller,R.B.; Chen,C. A stability-indicating HPLC method for the determination of benzalkonium chloride in phenylephrine HCl 10% ophthalmic solution, *J.Liq.Chromatogr.*, **1995**, 18, 553-563.

SAMPLE

Matrix: formulations

Sample preparation: Dilute 0.5% ophthalmic solution 1:5 with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 \times 4.6 3 μm CPS Hypersil-1 cyano

Mobile phase: MeCN:buffer 65:35 (Buffer was 50 mM sodium propionate adjusted to pH 5.3 with concentrated sulfuric acid.)

Flow rate: 1.3

Injection volume: 100

Detector: UV 214

CHROMATOGRAM

Retention time: 10.0 (C12), 11.7 (C14)

KEY WORDS

ophthalmic solutions; stability-indicating

REFERENCE

Parhizkari,G.; Delker,G.; Miller,R.B.; Chen,C. A stability-indicating HPLC method for the determination of benzalkonium chloride in 0.5% Tramadol ophthalmic solution, *Chromatographia*, **1995**, *40*, 155–158.

SAMPLE

Matrix: sewage

Sample preparation: Condition a C18ec SPE cartridge (Macherey-Nagel) with three bed volumes of MeOH and three bed volumes of water. Allow sewage to settle, add an 8 mL aliquot of the supernatant to the SPE cartridge, wash with 3 bed volumes of water, wash with 2 bed volumes of ethyl acetate, elute with 2 bed volumes of 1% calcium chloride in MeOH:ethyl acetate 50:50, inject a 15 μL aliquot of the eluate. (For all steps involving the SPE cartridge the flow rate should be 3 mL/min.)

HPLC VARIABLES

Column: 250 \times 4 5 μm Partisil PAC CN-NH₂

Mobile phase: Chloroform:MeOH 80:20

Column temperature: 15

Flow rate: 1

Injection volume: 15

Detector: F ex 383 em 459 following post-column extraction. The column effluent mixed with 37 $\mu\text{g/mL}$ sodium 9,10-dimethoxyanthracene-2-sulfonate in water pumped at 0.3 mL/min and the mixture flowed to a phase separator. The organic phase flowed to the detector and the aqueous phase was discarded. (9,10-Dimethoxyanthracene-2-sulfonate forms a hydrophobic fluorescent ion-pair with benzalkonium chloride.)

CHROMATOGRAM

Retention time: 2.49

Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: dimethyldidecylammonium chloride (DDMAC)

KEY WORDS

post-column reaction; post-column extraction; SPE

REFERENCE

Kümmerer,K.; Eitel,A.; Braun,U.; Hubner,P.; Daschner,F.; Mascart,G.; Milandri,M.; Reinthaler,F.; Verhoef,J. Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and high-performance liquid chromatography with post-column ion-pairing and fluorescence detection, *J.Chromatogr.A*, **1997**, *774*, 281–286.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm cyano

Mobile phase: MeCN:100 mM pH 5 sodium acetate buffer 60:40

Flow rate: 1

Detector: UV 254

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Prince,S.J.; Allen,L.V. Analysis of benzalkonium chloride and its homologs: HPLC vs HPCE (Abstract APQ 1093), *Pharm.Res.*, **1996**, *13*, S26–S26.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm cyano

Mobile phase: MeCN:100 mM pH 5 sodium acetate buffer 60:40

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 25 µg/mL

KEY WORDS

run time 25 min; for C12; C14; C16 homologs; comparison with capillary electrophoresis

REFERENCE

Prince,S.J.; McLaury,H.J.; Allen,L.V.,Jr. Comparison of HPCE and HPLC for the separation and quantitation of benzalkonium chloride homologs (Abstract 3012), *Pharm.Res.*, **1997**, *14*, S463–S464.

Benzbromarone

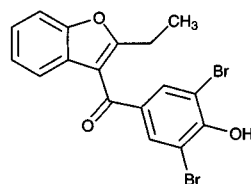
Molecular formula: C₁₇H₁₂Br₂O₃

Molecular weight: 424.09

CAS Registry No.: 3562-84-3

Merck Index: 1093

Lednicer No.: 2 354



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A: B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 204

CHROMATOGRAM

Retention time: 26.075

KEY WORDS

whole blood

REFERENCE

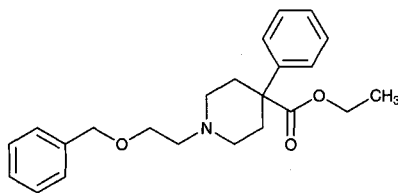
Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149-163.

Benzethidine

Molecular formula: $C_{23}H_{29}NO_3$

Molecular weight: 367.49

CAS Registry No.: 3691-78-9



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μ g/mL solution in MeOH, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 125 \times 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrizamide, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyrizamine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pectazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ran-

itidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191-225.

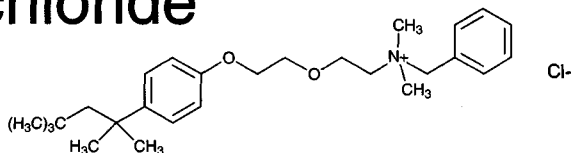
Benzethonium chloride

Molecular formula: $C_{27}H_{42}ClNO_2$

Molecular weight: 448.09

CAS Registry No.: 121-54-0

Merck Index: 1103



SAMPLE

Matrix: saliva

Sample preparation: Collect sample on Periopaper strip (filter paper), add paper to 100 μ L MeCN:water:glacial acetic acid 55:44.8:0.2 containing 7 mM sodium lauryl sulfate, vortex for 1 min, sonicate for 20 min, vortex, inject a 50 μ L aliquot.

HPLC VARIABLES

Column: 100 \times 2.1 5 μ m C18 ODS-B Exsil (HiChrome)

Mobile phase: MeCN:0.2% acetic acid 55:45 containing 5 mM sodium lauryl sulfate

Flow rate: 0.5

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 7

Internal standard: benzethonium

OTHER SUBSTANCES

Extracted: chlorhexidine

KEY WORDS

narrow-bore; benzethonium is IS

REFERENCE

Medlicott,N.J.; Ferry,D.G.; Tucker,I.G.; Rathbone,M.J.; Holborow,D.W.; Jones,D.S. High performance liquid chromatographic (HPLC) assay for the determination of chlorhexidine in saliva film, *J.Liq.Chromatogr.*, **1994**, 17, 1605-1620.

SAMPLE

Matrix: tissue

Sample preparation: Mix 10 g minced fish or squid + 50 mL 96% EtOH + 50 μ L 1-hexanesulfonic acid + 500 μ L 2 M HCl, mix thoroughly for 1 h, filter, extract residue with 50 mL 96% EtOH, filter, press filter cake, wash with 25 mL EtOH. Combine filtrates, evaporate under vacuum below 70°, take up residue in 500 μ L 2 M HCl + 500 μ L MeOH + 50 μ L 1-hexanesulfonic acid + 5 mL light petroleum (bp 30-40°), agitate for 2 min, pour out of flask, rinse flask with the same mixture, rinse flask with light petroleum. Combine all extracts, heat at 70° in a water bath until the organic layer disappears, add 2 mL dichloromethane:light petroleum 50:50, mix for 1 min, inject a 5-20 μ L aliquot of the organic phase.

HPLC VARIABLES

Column: 300 \times 3.9 μ Bondapak CN

Mobile phase: MeCN:100 mM ammonium acetate 75:25, containing 15 mL/L PIC B6 (Waters)

Flow rate: 1

Injection volume: 5-20

Detector: UV 254

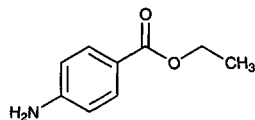
CHROMATOGRAM**Retention time:** 5.5**Limit of detection:** 5-10 ppm

KEY WORDSfish; squid

REFERENCE

Reuvers, T.B.A.; Ortiz, G.; Ramos, M.; Martín de Pozuelo, M. Rapid high-performance liquid chromatographic method for the determination of benzethonium chloride residues in fish products; confirmation by thin-layer chromatography, *J.Chromatogr.*, **1989**, 467, 321-326.

Benzocaine



Molecular formula: C₉H₁₁NO₂

Molecular weight: 165.19

CAS Registry No.: 94-09-7

Merck Index: 1116

Lednicer No.: 1 9

SAMPLE

Matrix: bile, blood, tissue

Sample preparation: Plasma, bile. Add three 250 μ L portions of MeOH to 250 μ L plasma and three 150 μ L portions of MeOH to 150 μ L bile, centrifuge. Remove MeOH from the supernatant using vacuum centrifugal evaporation. Dilute the residue to 1 mL with sample solvent. Filter (0.22 μ m) and inject a 100 μ L aliquot. Tissue. Homogenize 1.1 g white muscle, 640 mg liver, 250 mg trunk, or 170 mg head kidneys with 5 volumes of 100 mM acetic acid. Homogenize 500 mg red muscle and 320 mg skin with 5 volumes of 100 mM and 25 mM sodium acetate at pH 4.5, respectively. Add five volumes of MeOH to the homogenates, shake horizontally for 15 min. Centrifuge at 3000 g at 15° for 15 min and remove MeOH using vacuum centrifugal evaporation. Acidify the residue with 10% acetic acid to a final acetic acid concentration of 1% (skin to 2.5% acetic acid concentration) and add to a 3 mL Cyanopropyl (Supelco) SPE cartridge, elute with MeOH. Evaporate the MeOH under nitrogen at 40°. Reconstitute the residue with 500 μ L sample solvent. Filter (0.22 μ m) and inject a 100 μ L aliquot. (Sample solvent was MeOH:water:acetic acid 15:84:1.)

HPLC VARIABLES

Guard column: 10 \times 4.6 5 μ m Lichrosorb Diol

Column: 150 \times 4.6 3 μ m LC-18-DB Supelco

Mobile phase: Gradient. A was MeOH:water:acetic acid 5:94:1. B was MeOH:water:acetic acid 55:44:1. A:B 100:0 for 3 min, to 0:100 over 5 min, maintain at 0:100 for 15 min, return to 100:0 over 1 min.

Injection volume: 100

Detector: UV 286

CHROMATOGRAM

Retention time: 15.5

Limit of quantitation: 1 nM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; catfish; head kidney; liver; plasma; red muscle; skin; trunk; white muscle; kidney; muscle

REFERENCE

Szoke, A.; Hayton, W.L.; Schultz, I.R. Quantification of benzocaine and its metabolites in channel catfish tissues and fluids by HPLC, *J.Pharm.Biomed.Anal.*, **1997**, 16, 69–75.

SAMPLE

Matrix: blood

Sample preparation: Centrifuge 300 μ L whole blood at 5100 RCF for 8 min at 0°. Separate plasma from erythrocytes within 30 min after withdrawing blood. Warm sample to room temperature. Vortex 130 mg (125 μ L) plasma with 250 μ L MeOH for 1 min, centrifuge at 10 400 RCF for 15 min at 5°. Filter (0.45 μ PTFE) supernatant. Inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 23 × 4.0 5 µm spherical C18 with 17% carbon load, ODS type A (YMC, Inc., Wilmington)

Column: 150 × 4.6 5 µm spherical C18 with 17% carbon load, ODS type A (YMC, Inc., Wilmington)

Mobile phase: MeOH: 25 mM KH₂PO₄ 50:50

Flow rate: 1

Injection volume: 20

Detector: UV 286

CHROMATOGRAM

Retention time: 5.9

Limit of detection: 10 ng/mL

Limit of quantitation: 37 ng/mL

OTHER SUBSTANCES

Extracted: ethyl p-acetamidobenzoate

KEY WORDS

plasma; trout

REFERENCE

Bernardy, J.A.; Coleman, K.S.; Stehly, G.R.; Gingerich, W.H. Determination of benzocaine in rainbow trout plasma, *JAOAC Int.*, **1996**, 79, 623–627.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH₂PO₄ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 291

CHROMATOGRAM

Retention time: 3.51

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebuto-

lol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenylcyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loperazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, 1995, 40, 254-262.

SAMPLE

Matrix: bulk

Sample preparation: Prepare a 750 µg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 µm), inject a 15 µL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 µm LiChrospher 100

Column: 125 × 4 3 µm Spherisorb ODS-1

Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.

Flow rate: 0.7

Injection volume: 15

Detector: UV 210

CHROMATOGRAM

Retention time: 15.9

OTHER SUBSTANCES

Simultaneous: acetaminophen, acetylcodeine, caffeine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE

Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, 692, 121–129.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out 50 mg formulation, add 5 mL 1.5 mg/mL benzophenone in MeOH, make up to 50 mL with MeOH. Dilute 1 mL of this solution to 10 mL with MeOH, filter (0.45 μ m PTFE membrane), inject a 10 μ L aliquot.

HPLC VARIABLES

Guard column: 30 mm long Brownlee guard column

Column: 220 \times 4.6 5 μ m C18 (Brownlee)

Mobile phase: MeCN:water 60:40

Flow rate: 2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 1.8

Internal standard: benzophenone (3.3)

Limit of quantitation: 10000 ng/mL

OTHER SUBSTANCES

Simultaneous: benzyl benzoate

KEY WORDS

dermatological preparations

REFERENCE

Gigante,B.; Barros,A.M.V.; Teixeira,A.; Marcelo-Curto,M.J. Separation and simultaneous high-performance liquid chromatographic determination of benzocaine and benzyl benzoate in a pharmaceutical preparation, *J.Chromatogr.*, **1991**, 549, 217–220.

SAMPLE

Matrix: formulations

Sample preparation: Shake 2 g powder with 10 mL EtOH for 1 h, directly inject 1 mL of this solution using a syringe-coupled nylon filter (Teknokroma).

HPLC VARIABLES

Column: 125 \times 4 5 μ m Aluspher RP Select B (E. Merck) (alumina particles bonded with polybutadiene)

Mobile phase: MeOH:water:diammonium phosphate 30:70:0.2 (v:v:w), pH 8.2

Flow rate: 1

Injection volume: 20

Detector: UV 191

CHROMATOGRAM

Retention time: 3

OTHER SUBSTANCES

Simultaneous: menthol, tyrothricin

Noninterfering: gramicidin

REFERENCE

Caraballo,I.; Fernandez-Arevalo,M.; Holgado,M.-A.; Vela,M.-T.; Rabasco,A.-M. A rapid HPLC method for the quantification of tyrothricin, menthol, and benzocaine in pharmaceutical formulations, *J.Pharm.Sci.*, **1994**, 83, 1147–1149.

SAMPLE

Matrix: formulations

Sample preparation: Grind (Sorvall Omni-Mixer) and mix 14 lozenges for 2 min. Add 6.3 g powder to 15 mL mobile phase, sonicate (Bandelin Sonorex K 52) for 30 min, filter (paper), make up filtrate to 25 mL, dilute 13-fold, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Ultrabase C18 (Scharlau)

Mobile phase: MeOH:10 mM pH 3.31 KH_2PO_4 75:25

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 3.5

OTHER SUBSTANCES

Simultaneous: tyrothricin

Noninterfering: menthol

KEY WORDS

lozenges

REFERENCE

Ortiz-Boyer,F.; Tena,M.T.; Luque de Castro,M.D.; Valcárel,M. Development and validation of chromatographic methods (HPLC and GC) for the determination of the active components (benzocaine, tyrothricin and menthol) of a pharmaceutical preparation, *J.Pharm.Biomed.Anal.*, **1995**, 13, 1297–1303.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 5 μ L aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6

Flow rate: 2

Injection volume: 5

Detector: UV 305

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: butamben, lidocaine, pramoxine, procaine, tetracaine

REFERENCE

Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, 70, 569–570.

SAMPLE**Matrix:** solutions**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.**HPLC VARIABLES****Column:** 125 × 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V**CHROMATOGRAM****Retention time:** 1.0**OTHER SUBSTANCES**

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocetamine, benzphetamine, benquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinamine, sotalol, tacrine, terazosine, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 10.5

OTHER SUBSTANCES

Simultaneous: butacaine, lidocaine, bupivacaine, tetracaine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Supelcosil LC-ABZ

Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65

Flow rate: 1.5

Injection volume: 25

Detector: UV 254

CHROMATOGRAM

Retention time: 4.262

OTHER SUBSTANCES

Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, 12(3), 18–21.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisquinone, desipramine, dexamethasone, dextromethorphan, dextro-propoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylpyrrol, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, rescinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleppamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

Flow rate: 0.6

Injection volume: 25

Detector: UV 229

CHROMATOGRAM

Retention time: 6.25 (A), 6.23 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, lorazepam, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephentermine, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, mocllobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozone, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfapyrazole, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

also details of plasma extraction

REFERENCE

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103-119.

SAMPLE

Matrix: sunscreen

Sample preparation: Weigh out 1 g sunscreen, add 2-10 mL mobile phase, stir magnetically for 5 min, filter (0.45 μ m Millex-HV), inject an aliquot.

HPLC VARIABLES

Column: 200 \times 5 μ m Nucleosil C18

Mobile phase: MeCN:15 mM phosphoric acid 18:82

Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM

Limit of detection: 500 ng/mL

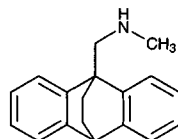
OTHER SUBSTANCES

Extracted: p-aminobenzoic acid

REFERENCE

Bruze,M.; Gruvberger,B.; Thulin,I. PABA, benzocaine, and other PABA esters in sunscreens and after-sun products, *Photodermatol.Photoimmunol.Photomed.*, **1990**, 7, 106–108.

Benzoctamine



Molecular formula: C₁₈H₁₉N

Molecular weight: 249.36

CAS Registry No.: 17243-39-9, 10085-81-1 (HCl)

Merck Index: 1117

Lednicer No.: 2 220

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

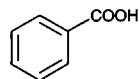
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscaphine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Benzoic acid



Molecular formula: C₇H₆O₂

Molecular weight: 122.12

CAS Registry No.: 65-85-0

Merck Index: 1122

SAMPLE

Matrix: beverage

Sample preparation: Sonicate 25 mL beverage for 15-20 min, filter (0.45 µm) if necessary, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeCN:MeOH:water:acetic acid 10:20:70:1

Flow rate: 1.5

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 12

OTHER SUBSTANCES

Simultaneous: hydroquinine, quinine, saccharin

KEY WORDS

tonic water; soft drinks

REFERENCE

Valenti,L.P. Liquid chromatographic determination of quinine, hydroquinine, saccharin, and sodium benzoate in quinine beverages, *J.Assoc.Off.Anal.Chem.*, **1985**, 68, 782-784.

SAMPLE

Matrix: beverages

Sample preparation: Filter sample.

HPLC VARIABLES

Column: 150 × 4.5 5 µm Hiasil C18 (Higgins)

Mobile phase: MeOH:25 mM phosphate buffer 45:55, pH 3.0

Flow rate: 1.0

Injection volume: 20

Detector: UV 218

CHROMATOGRAM

Retention time: 6.0

Limit of detection: 0.57 mg/mL

OTHER SUBSTANCES

Simultaneous: aspartame, caffeine

KEY WORDS

comparison with UV spectrophotometry and capillary electrophoresis; soft drinks

REFERENCE

McDevitt,V.L.; Rodriguez,A.; Williams,K.R. Analysis of soft drinks: UV spectrophotometry, liquid chromatography, and capillary electrophoresis, *J.Chem.Educ.*, **1998**, 75, 625-629.

SAMPLE**Matrix:** beverages, syrup**Sample preparation:** Dilute syrup ten fold. Filter (0.45 μm) beverages and diluted syrup, inject a 10-20 μL aliquot of the filtrate.

HPLC VARIABLES**Column:** 300 \times 3.9 10 μm μ Bondapak C18**Mobile phase:** MeOH:acetic acid:water 20:5:75**Flow rate:** 2**Injection volume:** 10-20**Detector:** UV 254

CHROMATOGRAM**Retention time:** 9.5**Limit of detection:** 100 ng

OTHER SUBSTANCES**Simultaneous:** acesulfame, caffeine, dulcin, p-hydroxybenzoic acid, saccharin, vanillin

REFERENCEVeerabhadrarao,M.; Narayan,M.S.; Kapur,O.; Sastry,C.S. Reverse phase liquid chromatographic determination of some food additives, *J.Assoc.Off.Anal.Chem.*, **1987**, 70, 578-582.

SAMPLE**Matrix:** bile, blood**Sample preparation:** Blood, plasma. 250 μL Blood or plasma + 50 μL 16 $\mu\text{g/mL}$ methoxybenzoic acid in water + 800 μL MeCN, mix, centrifuge. Remove the supernatant and dry it under a stream of nitrogen, reconstitute the residue in 200 μL MeCN:0.5% acetic acid 10:90, centrifuge, inject an aliquot. Bile. Inject bile directly.

HPLC VARIABLES**Guard column:** 22 \times 3.4 37-50 μm C18 (Waters)**Column:** 300 \times 3.9 10 μm Ultrasphere**Mobile phase:** Gradient. MeCN:0.5% acetic acid 10:90 for 10 min then to 72.5:27.5 over 2 min, maintain at 72.5:27.5 for 8 min, return to initial conditions over 1 min, re-equilibrate for 4 min.**Flow rate:** 1**Detector:** UV 254

CHROMATOGRAM**Retention time:** 17.8**Internal standard:** methoxybenzoic acid (19.1)

OTHER SUBSTANCES**Extracted:** hippuric acid, benzoyl glucuronide

KEY WORDS

plasma

REFERENCEChiba,M.; Poon,K.; Hollands,J.; Pang,K.S. Glycine conjugation activity of benzoic acid and its acinar localization in the perfused rat liver, *J.Pharmacol.Exp.Ther.*, **1994**, 268, 409-416.

SAMPLE**Matrix:** blood**Sample preparation:** Condition a 100 mg Bond Elut C18 SPE cartridge with 2 mL MeCN and 2 mL pH 3 aqueous acetic acid. Dilute 1 mL serum with 1 mL pH 3 aqueous acetic

acid, add to SPE cartridge, wash with 500 μ L pH 3 aqueous acetic acid, elute with 1 mL MeCN acidified to pH 2.5 with acetic acid, inject a 20 μ L aliquot of the eluate.

HPLC VARIABLES

Column: Capcell Pak C-18 AG-120 (Shiseido)

Mobile phase: MeCN:water 20:80

Flow rate: 1.2

Injection volume: 20

Detector: UV 250

CHROMATOGRAM

Retention time: 5.8

KEY WORDS

serum; dental material; methyl methacrylate polymer; plexiglass; horse; SPE

REFERENCE

Shintani,H.; Tsuchiya,T.; Hata,Y.; Nakamura,A. Solid phase extraction and HPLC analysis of toxic components eluted from methyl methacrylate dental materials, *J.Anal.Toxicol.*, **1993**, *17*, 73-78.

SAMPLE

Matrix: bulk, formulations

Sample preparation: 100 mg Bulk drug or formulation containing 100-120 mg drug + 10 mL 1 (bulk) or 4 (formulations) mg/mL benzoic acid in MeOH:water 50:50 + 20 mL 5 mg/mL p-nitroacetophenone in MeOH, make up to 100 mL with water, inject an aliquot.

HPLC VARIABLES

Column: 300 \times 4 10 μ m μ Bondapak C18

Mobile phase: MeCN:MeOH:water:glacial acetic acid 20:5:74:1 containing 0.05-0.08% sodium 1-heptanesulfonate, pH 3.1

Flow rate: 2

Injection volume: 5

Detector: UV 278

CHROMATOGRAM

Retention time: 6

Internal standard: benzoic acid, p-nitroacetophenone (12)

OTHER SUBSTANCES

Simultaneous: 4-amino-2-chlorobenzoic acid, impurities, chloroprocaine

KEY WORDS

benzoic acid is IS

REFERENCE

Menon,G.; Norris,B.; Webster,J. Simultaneous determination of chloroprocaine hydrochloride and its degradation product 4-amino-2-chlorobenzoic acid in bulk drug and injection solutions by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 251-253.

SAMPLE

Matrix: food

Sample preparation: 5 g Food + 2 mL 1 mg/mL 4-hydroxyacetanilide in MeOH, make up to 15 mL with MeOH, mix, centrifuge at 1500 g for 10 min, filter an aliquot of the supernatant (0.45 μ m), inject a 15 μ L aliquot.

HPLC VARIABLES

Guard column: RP18 (Brownlee)

Column: 10 μ m Spheri RP-18 (Brownlee)

Mobile phase: MeOH:30 mM pH 6.5 phosphate buffer 5:95

Flow rate: 2

Injection volume: 15

Detector: UV 227

CHROMATOGRAM

Retention time: 4.24

Internal standard: 4-hydroxyacetanilide (14.57)

OTHER SUBSTANCES

Simultaneous: sorbic acid

KEY WORDS

beverages; fruit; seafood; vegetables; sauces; dairy products

REFERENCE

Bui,L.V.; Cooper,C. Reverse-phase liquid chromatographic determination of benzoic and sorbic acids in foods, *J.Assoc.Off.Anal.Chem.*, **1987**, 70, 892–896.

SAMPLE

Matrix: food

Sample preparation: 2 g Soy sauce or 1 g sugared fruit or roast beef + 10 g NaCl, make up to 50 mL with acetone, swirl vigorously, let stand for 30 min, filter, wash the solid with acetone, make up filtrate to 50 mL with acetone, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m monomeric C18 (Shoko, Kyoto)

Mobile phase: MeCN:50 mM pH 4.5 α -hydroxyisobutyric acid in water 22:34 containing 2.5 mM hexadecyltrimethylammonium bromide

Flow rate: 1

Injection volume: 20

Detector: UV 233

CHROMATOGRAM

Retention time: 24

OTHER SUBSTANCES

Simultaneous: acesulfame-K, 3-t-butyl-4-hydroxyanisole, butyl p-hydroxybenzoate, t-butylhydroxyquinone, dulcin, ethyl p-hydroxybenzoate, isobutyl p-hydroxybenzoate, isopropyl p-hydroxybenzoate, methyl p-hydroxybenzoate, saccharin, sodium dehydroacetate, sorbic acid

KEY WORDS

soy sauce; roast beef; sugared fruit

REFERENCE

Chen,B.H.; Fu,S.C. Comparison of extraction methods and column types for the determination of additives by liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, 19, 625–643.

SAMPLE

Matrix: formulations

Sample preparation: 5 mL Formulation + 5 mL IS solution, make up to 50 mL with MeOH, inject 10 μ L aliquot. (IS solution was 0.2 mg p-nitroacetophenone and 2.5 mg isobutyrophenone per mL of MeOH.)

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18

Mobile phase: MeCN:water:reagent 25:60:15, pH 2.6 (Reagent was MeOH containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate.)

Flow rate: 2

Injection volume: 10

Detector: UV 257

CHROMATOGRAM

Retention time: 3.5

Internal standard: p-nitroacetophenone (6) and isobutyrophenone (13)

OTHER SUBSTANCES

Simultaneous: benzyl alcohol, hydroxyzine, benzaldehyde, p-chlorobenzoic acid, p-chlorobenzaldehyde, p-chlorobenzophenone

KEY WORDS

injections; stability-indicating

REFERENCE

Menon, G.N.; Norris, B.J. Simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol in injection solutions by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, 70, 697–698.

SAMPLE

Matrix: formulations

Sample preparation: Leach 200 or 300 mg ground capsule or tablet with water or mobile phase and dilute to 50 mL, sonicate for 5 min, centrifuge at 2500 rpm for 5 min, inject an aliquot. Dilute 4–25 mL of liquid formulations to 250 mL with water, inject an aliquot.

HPLC VARIABLES

Column: Partisil-10 C8

Mobile phase: MeOH:MeCN:water:PIC-B5 50:170:755:25 (PIC-B5 (Waters) is 200 mM sodium pentanesulfonate in glacial acetic acid.)

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 14.5

OTHER SUBSTANCES

Simultaneous: phenylephrine, phenylpropanolamine, guaifenesin, impurities, degradation products

KEY WORDS

tablets; capsules; liquid formulations; stability-indicating

REFERENCE

Schieffer, G.W.; Smith, W.O.; Lubey, G.S.; Newby, D.G. Determination of the structure of a synthetic impurity in guaifenesin: modification of a high-performance liquid chromatographic method for phenylephrine hydrochloride, phenylpropanolamine hydrochloride, guaifenesin, and sodium benzoate in dosage forms, *J.Pharm.Sci.*, **1984**, 73, 1856–1858.

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeOH:buffer 20:80 (Buffer was 15 mM 1-butanesulfonic acid + 15 mM KH_2PO_4 + 2 mL/L triethylamine, pH adjusted to 4.8 ± 0.1 with dilute phosphoric acid.)

Column temperature: 50

Flow rate: 2

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 3.9

OTHER SUBSTANCES

Simultaneous: codeine, acetaminophen, p-aminophenol, codeine N-oxide, codeinone

KEY WORDS

elixir; stability-indicating

REFERENCE

Sisco, W.R.; Rittenhouse, C.T.; Everhart, L.A.; McLaughlin, A.M. Simultaneous high-performance liquid chromatographic stability-indicating analysis of acetaminophen, codeine phosphate, and sodium benzoate in elixirs, *J.Chromatogr.*, **1986**, 354, 355–366.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out 2 g metronidazole benzoate suspension containing 3.5% metronidazole benzoate, add 80 mL MeOH:water 80:20, sonicate for a few minutes, make up to 100 mL with MeOH water 80:20, centrifuge at 900 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 2 30-38 μm Whatman Co:Pell

Column: 250 × 4.6 10 μm Perkin-Elmer C18

Mobile phase: MeOH:water 60:40 containing 5 mM acetate buffer and 50 mM KNO_3 , pH^* 5.2

Flow rate: 1

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: metronidazole, metronidazole benzoate, methylparaben, propylparaben

KEY WORDS

suspensions

REFERENCE

Pashankov, P.P.; Kostova, L.L. Reversed-phase high-performance liquid chromatography of metronidazole benzoate in suspension dosage form, *J.Chromatogr.*, **1987**, 394, 382–387.

SAMPLE

Matrix: formulations

Sample preparation: Dilute 1 mL syrup to 50 mL with mobile phase, filter (0.45 μm), inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax CN

Mobile phase: MeCN:water:formic acid:methanesulfonic acid 500:500:1:1, pH adjusted to 3.5 with 10% NaOH

Flow rate: 1

Injection volume: 20

Detector: UV 290

CHROMATOGRAM

Retention time: 3

OTHER SUBSTANCES

Simultaneous: guaifenesin, saccharin, dextromethorphan

KEY WORDS

syrup

REFERENCE

Chen,T.M.; Pacifico,J.R.; Daly,R.E. High-pressure liquid chromatographic assay of dextromethorphan hydrobromide, guaifenesin, and sodium benzoate in an expectorant syrup, *J.Chromatogr.Sci.*, **1988**, 26, 636-639.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out ground tablets or capsules equivalent to 150 mg bepridil.HCl, add 150 mL MeCN, shake for 30 min, dilute to 200 mL with MeCN, filter (Schleicher & Schüll paper, grade 588), inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 10 µm µBondapak C18

Mobile phase: MeCN:buffer 580:405 (Buffer was 1.1 g sodium 1-heptanesulfonate in 405 mL water, adjust to pH 2.37 with glacial acetic acid (ca. 15 mL).)

Column temperature: 35

Flow rate: 1.3

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 2.6

OTHER SUBSTANCES

Simultaneous: impurities, benzaldehyde, N-benzylaniline, bepridil

KEY WORDS

capsules; tablets

REFERENCE

Renzi,N.L.; Fronheiser,M.E.; Duong,H.T.; Fulton,D.J.; Rabinowitz,M. Stability-indicating high-performance liquid chromatography assay for bepridil hydrochloride drug substance and drug products, *J.Chromatogr.*, **1989**, 462, 398-405.

SAMPLE

Matrix: formulations

HPLC VARIABLES

Column: C18

Mobile phase: MeCN:buffer 13:87 (Buffer was 0.01% acetic acid containing 0.001% sodium octanesulfonate.)

Flow rate: 2.5

Detector: UV 280

CHROMATOGRAM

Internal standard: benzoic acid

OTHER SUBSTANCES

Simultaneous: dopamine

KEY WORDS

injections; 5% dextrose; benzoic acid is IS

REFERENCE

Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

SAMPLE

Matrix: formulations

Sample preparation: Dilute 10 mL to 1 L with water.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μ Bondapak C18

Mobile phase: MeCN:water:diethylamine:glacial acetic acid 250:739:1:10, apparent pH 4.1

Column temperature: 35

Flow rate: 1.3

Injection volume: 25

Detector: UV 273

CHROMATOGRAM

Retention time: 5.3

OTHER SUBSTANCES

Simultaneous: guaifenesin, dextromethorphan

KEY WORDS

liquid formulations; stability-indicating

REFERENCE

Wilson,T.D.; Jump,W.G.; Neumann,W.C.; San Martin,T. Validation of improved methods for high-performance liquid chromatographic determination of phenylpropanolamine, dextromethorphan, guaifenesin and sodium benzoate in a cough-cold formulation, *J.Chromatogr.*, **1993**, *641*, 241–248.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out 4 mL of 1 mg/mL solution and make up to 100 mL with mobile phase, inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax cyano special

Mobile phase: MeCN:buffer 50:50 (Buffer was 1% triethylamine, pH adjusted to 6 with conc phosphoric acid.)

Flow rate: 1

Injection volume: 20

Detector: UV 215

CHROMATOGRAM**Retention time:** 4

OTHER SUBSTANCES**Simultaneous:** fluoxetine

KEY WORDSsyrup; elixir

REFERENCE

Peterson, J.A.; Risley, D.S.; Anderson, P.N.; Hostettler, K.F. Stability of fluoxetine hydrochloride in fluoxetine solution diluted with common pharmaceutical diluents, *Am.J.Hosp.Pharm.*, **1994**, *51*, 1342–1345.

SAMPLE**Matrix:** juice

Sample preparation: Condition a Sep-Pak Classic SPE cartridge with 2 mL MeOH and 5 mL water. Centrifuge 10 mL orange juice at 1500 g for 5 min, add 1 mL supernatant to the SPE cartridge, wash with 3 mL hexane:MeCN 98:2, force air through cartridge three times, elute with 3 mL MeOH, make eluate up to 3 mL with MeOH, filter (0.45 μ m)

HPLC VARIABLES**Guard column:** PRP-1 (Hamilton)**Column:** 250 \times 4.1 10 μ m PRP-1 (Hamilton)

Mobile phase: MeCN:buffer 40:60 (Buffer was 6.8 g KH_2PO_4 in 1 L water, adjust pH to 2.3 with 85% phosphoric acid.)

Flow rate: 0.7**Injection volume:** 10**Detector:** UV 230

CHROMATOGRAM**Retention time:** 7.26**Limit of detection:** 0.5 ppm

KEY WORDSorange; SPE

REFERENCE

Lee, H.S. Liquid chromatographic determination of benzoic acid in orange juice: Interlaboratory study, *J.AOAC Int.*, **1995**, *78*, 80–82.

SAMPLE**Matrix:** perfusate

HPLC VARIABLES**Column:** Lichrospher 100 RP-18**Mobile phase:** MeOH:water containing 10 mM citric acid 45:55**Column temperature:** 45**Flow rate:** 1**Detector:** UV 230

KEY WORDSrat; intestine; Caco-2 monolayer

REFERENCE

Takagi, M.; Taki, Y.; Sakane, T.; Nadai, T.; Sezaki, H.; Oku, N.; Yamashita, S. A new interpretation of salicylic acid transport across the lipid bilayer: Implication of pH-dependent but not carrier-mediated absorption from the gastrointestinal tract, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 1175–1180.

SAMPLE**Matrix:** solutions

Sample preparation: Add 500 μL of a solution in MeCN to 100 mg finely powdered potassium carbonate, add 250 μL 3.8 mM 18-crown-6 in MeCN, add 250 μL 0.8 mM reagent in MeCN, heat at 80° in the dark for 20 min, cool, inject a 5 μL aliquot. (Synthesize the reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone, as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 \times 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 \times 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromo-methyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°).)

HPLC VARIABLES**Column:** 100 \times 4 10 μm Radial-Pak C18 (Waters)**Mobile phase:** Gradient. MeOH:water from 57:43 to 100:0 over 20 min, maintain at 100:0 for 12 min**Flow rate:** 2**Injection volume:** 5**Detector:** F ex 370 em 450

CHROMATOGRAM**Retention time:** 9.4**Limit of detection:** 0.3-1 fmole

OTHER SUBSTANCES

Simultaneous: p-aminobenzoic acid, arachidic acid, arachidonic acid, butyric acid, capric acid, caproic acid, caprylic acid, deoxyuridine, glucuronic acid, imidazole-4-acetic acid, lauric acid, linoleic acid, linolenic acid, margaric acid, 1-methyl-4-imidazoleacetic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, propionic acid, salicylic acid, stearic acid, thymidine, uridine, valeric acid

KEY WORDS

derivatization

REFERENCE

Yamaguchi,M.; Hara,S.; Matsunaga,R.; Nakamura,M.; Ohkura,Y. 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *J.Chromatogr.*, **1985**, 346, 227-236.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58

Flow rate: 1.5

Injection volume: 10

Detector: UV 261

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: ascorbic acid, salicylic acid, quinine, dihydroquinine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403-418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare an aqueous solution, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Nucleosil C18

Mobile phase: MeCN:MeOH:buffer:triethylamine 4:4:92:0.01 (Buffer was 0.05% sodium octanesulfonate adjusted to pH 2.2 with 3 M phosphoric acid.)

Flow rate: 1.5

Injection volume: 10

Detector: UV 215

CHROMATOGRAM

Retention time: 2.4

OTHER SUBSTANCES

Simultaneous: p-aminobenzoic acid, benzaldehyde, benzyl alcohol, protirelin

REFERENCE

Rao,G.N.; Sutherland,J.W.; Menon,G.N. High-performance liquid chromatographic assay for thyrotropin releasing hormone and benzyl alcohol in injectable formulation, *Pharm.Res.*, **1987**, 4, 38-41.

SAMPLE

Matrix: solutions

Sample preparation: React the carboxylic acid, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in MeCN at 45° for 2 h, inject a 10 µL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for

12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°.)

HPLC VARIABLES

Column: 250 × 4 7 µm RP-18 LiChrocart (Merck)

Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42

Flow rate: 1

Injection volume: 10

Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.6 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 5

Limit of detection: 1 pmole

OTHER SUBSTANCES

Simultaneous: quinoxaline-2-carboxylic acid, salicylic acid

KEY WORDS

derivatization

REFERENCE

Munns, R.K.; Roybal, J.E.; Shimoda, W.; Hurlbut, J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J. Chromatogr.*, 1988, 442, 209-218.

SAMPLE

Matrix: solutions

Sample preparation: Inject 50 µL onto column A in series with column B, after 1.1 min switch so that column A comes after column B, continue to elute.

HPLC VARIABLES

Column: A 15 × 3.2 7 µm New Guard RP-18 (Applied Biosystems); B 100 × 4.6 3 µm Econosphere C18 (Alltech)

Mobile phase: MeCN:water:phosphoric acid 25:75:0.2

Flow rate: 1

Injection volume: 50

Detector: UV 200

CHROMATOGRAM

Retention time: 6

Limit of detection: 0.05 ppm

OTHER SUBSTANCES

Extracted: toluene, cresol, phenol

KEY WORDS

groundwater; water; column-switching

REFERENCE

Chamkasem,N.; Hill,K.D.; Sewell,G.W. High-performance liquid chromatographic column-switching technique for the determination of intermediates of anaerobic degradation of toluene in ground water microcosm, *J.Chromatogr.*, **1991**, 587, 185-191.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μ L aliquot of a 5-1000 nM solution of a carboxylic acid in MeCN with 100 μ L 18-crown-6 solution, add 100 μ L 100 μ M dansyl-BAP in MeCN, mix, let stand at room temperature (aliphatic acids) or 55° (aromatic acids) for 30 min, add 100 μ L 3 mM thymine in MeCN, add 5 mg potassium bicarbonate, vortex for 30 s, let stand for 30 min, evaporate to dryness under a stream of nitrogen. Reconstitute with dichloromethane, add to a Bond-Elut silica SPE cartridge, elute with 1.5 mL MeCN:dichloromethane 50:50. Evaporate the eluate to dryness and reconstitute the residue with 500 μ L mobile phase, inject a 25 μ L aliquot. (Prepare 18-crown-6 solution by sonicating a 1 mg/mL solution of 18-crown-6 in MeCN containing 1 mg/mL potassium bicarbonate for 20 min. Prepare dansyl-BAP (N-(bromoacetyl)-N'-[5-(dimethylamino)naphthalene-1-sulfonyl]piperazine) as follows. Slowly add a solution of 135 mg dansyl chloride in 30 mL acetone to a 10-fold molar excess of piperazine in acetone:water 75:25, stir at 50° for 1 h, evaporate the acetone. Acidify the remaining aqueous layer with concentrated nitric acid, wash 3 times with 15 mL portions of dichloromethane, adjust the pH of the aqueous layer to 11 with concentrated NaOH, extract three times with 15 mL portions of dichloromethane. Combine the extracts and dry them over anhydrous calcium chloride, concentrate to about 5 mL, chromatograph on a 400 \times 25 column of 60-200 μ m silica gel Si-60, wash with about 20 mL dichloromethane:MeOH 99:1 to remove a small fluorescent band, elute with about 30 mL dichloromethane:MeOH 95:5-94:6 to obtain a solution of dansyl-piperazine, determine the concentration by UV absorption at 340 nm (extinction coefficient = 4300 in MeOH). Stir a solution of 700 mg bromoacetic acid and 1.1 g dicyclohexylcarbodiimide in 100 mL MeCN at room temperature for 1 min, slowly add 470 μ moles dansylpiperazine in MeCN, stir for 1 h, evaporate to dryness, reconstitute the residue with 10 mL dichloromethane, filter. Chromatograph the filtrate on a 400 \times 25 column of 40-60 μ m Si-60 silica gel with dichloromethane, when the first-eluting, strongly-fluorescent yellow band reaches the outlet change the eluent to dichloromethane:MeOH 99:1, collect about 50 mL eluate to obtain dansyl-BAP.)

HPLC VARIABLES

Column: 150 \times 3.1 5 μ m LiChrosorb RP-18

Mobile phase: MeCN:water 60:40, containing 2.5 mM pH 7.0 imidazole buffer

Flow rate: 0.5

Injection volume: 25

Detector: F ex 246 em 490 (cut-off filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN containing 5 mM bis(2-nitrophenyl)oxalate pumped at 0.3 mL/min and the mixture flowed immediately to the detector. (Prepare bis(2-nitrophenyl)oxalate by dissolving 13.9 g 2-nitrophenol in 250 mL benzene (Caution! Benzene is a carcinogen!), remove 50 mL benzene by azeotropic distillation, cool to 10°, add 10.1 g freshly distilled triethylamine, add 7 g oxalyl chloride dropwise, allow to warm to room temperature, let stand overnight, evaporate to dryness under reduced pressure, recrystallize to give bis(2-nitrophenyl)oxalate (*J. Chem. Educ.* 1974, 51, 529).)

CHROMATOGRAM

Retention time: 24

Limit of detection: 0.8-1 pmole

OTHER SUBSTANCES

Simultaneous: 2,4-dichlorobenzoic acid, ibuprofen, lipoic acid, 2-methoxybenzoic acid, naproxen, octanoic acid

KEY WORDS

derivatization; post-column reaction; SPE

REFERENCE

Kwakman,P.J.M.; Van Schaik,H.P.; Brinkman,U.A.T.; de Jong,G.J. *N*-(Bromoacetyl)-*N'*-[5-(dimethylamino)naphthalene-1-sulfonyl]piperazine as a sensitive labeling reagent for the determination of carboxylic acids by liquid chromatography with peroxyoxalate chemiluminescence and fluorescence detection, *Analyst*, **1991**, *116*, 1385–1391.

SAMPLE

Matrix: solutions

Sample preparation: Dilute a 10 mg/mL solution of caffeine in water 1:1000 with mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 2540 \times 4.6 5 μ m C18 (Supelco)

Mobile phase: MeOH:10 mM ammonium acetate and 2.5 mM sodium heptanesulfonate 20:80, pH adjusted to 5.1 with glacial acetic acid

Flow rate: 2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: caffeine

KEY WORDS

water

REFERENCE

Donnelly,R.F.; Tirona,R.G. Stability of citrated caffeine injectable solution in glass vials, *Am.J.Hosp.Pharm.*, **1994**, *51*, 512–514.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cor-

tisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephenetermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mi-bolone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1-10 µg/mL solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Hypersil SCX/C18

Mobile phase: MeCN:25 mM pH 3 Na₂HPO₄ 50:50

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.69

OTHER SUBSTANCES

Also analyzed: amitriptyline, barbital, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS

effect of mobile phase pH on capacity factor is discussed

REFERENCE

Walshe, M.; Kelly, M.T.; Smyth, M.R.; Ritchie, H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J. Chromatogr. A*, **1995**, 708, 31–40.

SAMPLE

Matrix: solutions

Sample preparation: Acidify 1 mL solution with 200 μ L 100 mM HCl, extract with ether. Evaporate the ether to dryness, reconstitute with 3 mL mobile phase, inject a 20 μ L aliquot.

HPLC VARIABLES

Guard column: 4 \times 4 Lichrosphere 100RP-18

Column: 250 \times 4 Lichrosphere 100RP-18

Mobile phase: MeCN:10 mM pH 6.8 phosphate buffer containing 10 mM sec-butylamine 5:95

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: F ex 305 em 407

CHROMATOGRAM

Retention time: 4.5

OTHER SUBSTANCES

Simultaneous: 4-aminosalicylic acid

REFERENCE

Motohashi, N.; Saito, Y. Rate constants for reaction of hydroxyl radicals with sulfapyridine and aminosalicylic acids, *Chem. Pharm. Bull.*, **1996**, 44, 163–166.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve salt of pindolol in MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES

Column: 200 \times 4.6 5 μ m Hypersil RP-18

Mobile phase: Gradient. MeOH:buffer from 20:80 to 80:20 over 10 min. (Buffer was 2% acetic acid containing 1.1% sodium 1-heptanesulfonate.)

Column temperature: 40

Flow rate: 1.5

Detector: UV 273

CHROMATOGRAM

Retention time: 5.9

OTHER SUBSTANCES

Simultaneous: 2-methoxyphenylacetic acid (UV 270), pindolol (UV 254)

REFERENCE

Pietiläinen,H.; Saesmaa,T. HPLC determination of pindolol benzoate and pindolol 2-methoxyphenyl-acetate, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, 19, 583–591.

SAMPLE

Matrix: urine

Sample preparation: Centrifuge urine at 2000 g for 10 min, filter (0.45 μm Millex-HV), dilute ten-fold with water, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 \times 3.9 4 μm Nova-Pak C18

Mobile phase: MeOH:25 mM pH 4.5 acetate buffer 5:95

Column temperature: 35

Flow rate: 1

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 10.4

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: phenylacetic acid, hippuric acid, phenaceturic acid

KEY WORDS

sheep

REFERENCE

Arín,M.J.; Díez,M.T.; Resines,J.A. Rapid and simple method for the determination of urinary benzoic and phenylacetic acids and their glycine conjugates in ruminants by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1992**, 582, 13–18.

SAMPLE

Matrix: vegetables

Sample preparation: Place 25 g homogenized vegetables in a flask and make up to 100 mL with MeOH:water 60:40, shake vigorously for 10 min, filter (paper). Centrifuge an aliquot of the filtrate at 11600 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 \times 4 Spherisorb ODS-2

Column: 250 \times 4 Spherisorb ODS-2

Mobile phase: 30 mM pH 6.7 Phosphate buffer (Buffer was 2.5 g $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ and 2.5 g KH_2PO_4 in 1 L water.)

Flow rate: 2

Injection volume: 20

Detector: UV 230

CHROMATOGRAM

Retention time: 2.5

Limit of detection: 1 ppm

OTHER SUBSTANCES

Extracted: sorbic acid

KEY WORDS

peppers; tomatoes; caperberries; onions

REFERENCE

Montaño,A.; Sánchez,A.H.; Rejano,L. Determination of benzoic and sorbic acids in packaged vegetable products. Comparative evaluation of methods, *Analyst*, **1995**, 120, 2483–2487.

SAMPLE

Matrix: wine

Sample preparation: Adjust pH of wine to 7-8 with potassium bicarbonate. Remove a 1 mL aliquot and add it to 1 mL 170 mM phenacyl bromide in acetone, add 1 mL 17 mM 18-crown-6 in acetone, add 1 mL acetone, heat in a boiling water bath for 75 min, cool, inject a 10 μ L aliquot. (Recrystallize phenacyl bromide from n-heptane.)

HPLC VARIABLES

Guard column: 37-50 μ m Bondapak C18/Corasil

Column: 250 \times 4 7 μ m RP-18 (Merck)

Mobile phase: Gradient. MeOH:water from 35:65 to 85:15 over 20 min.

Flow rate: 2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 14.7

OTHER SUBSTANCES

Extracted: acetic acid, anisic acid, benzoic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α -ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, salicylic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid, ascorbic acid

KEY WORDS

derivatization

REFERENCE

Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, 322, 177–189.

SAMPLE

Matrix: yogurt

Sample preparation: 500 mg Homogenized yogurt + 7 mL buffer, sonicate for 2 min, shake mechanically for 20 min, centrifuge at 2000 rpm for 10 min, repeat extraction twice more. Combine the supernatants and make up to 25 mL with buffer. Remove a 5 mL extract and add it to 5 mL 10 mM tri-n-octylamine in chloroform, shake for 20 min, centrifuge at 2000 rpm for 10 min. Remove 2.5 mL of the organic phase and add it to 2.5 mL 100 mM sodium perchlorate in water, extract, centrifuge, inject an aliquot of the aqueous phase. (Buffer was 24.650 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 1.260 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ made up to 2 L with water, pH 5.5.)

HPLC VARIABLES

Column: 250 \times 4 10 μ m RP-18 (Merck)

Mobile phase: MeOH:buffer 40:60 (Buffer was 900 μ L 1 M phosphoric acid and 27.598 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ made up to 2 L with water, pH 4.5.)

Injection volume: 100

Detector: UV 270 for 4 min then UV 240

CHROMATOGRAM

Retention time: 5

Limit of detection: 20 µg/g

OTHER SUBSTANCES

Simultaneous: saccharin, sorbic acid

REFERENCE

Puttemans,M.L.; Branders,C.; Dryon,L.; Massart,D.L. Extraction of organic acids by ion-pair formation with tri-n-octylamine. Part 6. Determination of sorbic acid, benzoic acid, and saccharin in yogurt, *J.Assoc.Off.Anal.Chem.*, **1985**, 68, 80–82.